Characterization of Hydroxyapatite in Biomimetic Solution

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***Abstract*— The aim of this study is to synthesis a biomimetic technique that synthesis a bone like-apatite in biomimetic solution and to optimize decomposition of hydroxyapatite (HAp) crystals by modification of ion concentration in simulated body fluid solution. HAp has a wide range of medical application due to its biological apatite condition. However, elevated processing conditions such as higher temperature have been report to produce hydroxyapatite with properties different from biological apatite. To overcome this limitation, the synthesis of hydroxyapatite involves the use of simulated body fluid with controlled parameter to growth hydroxyapatite at body temperature & physiological pH has been explore. The presence study was conducted using precipitation of HAp in biomimetic technique. HAp was synthesis in simulated body fluid with the presence of Ca and P precursor, dried at 80^{o}C and calcined at 800^{o}C for 2hr. The result shows that the hydroxyapatite produced was as B type carbonated HAp with well-formed crystallinity. However, the incubation period of calcination during HAp formation was not enough due to presence of calcium phosphate. This bone-like apatite can be used as material in bone structure and any medical area.**

Keywords*— *hydroxyapatite, biomimetic solution, simulated body fluid

I. INTRODUCTION

Hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ is a major constituent of natural bone that makes up of 70-80wt% of biological apatite. It is an essential ingredient of calcified hard tissue that comprise mineral phases such as enamel, dentin and bone. Nonetheless, calcified hard tissue or biological apatite has a discrete properties from pure hydroxyapatite in stoichiometry, composition and crystallinity. (Leena, Rana, Webster, & Ramalingam, 2016) (Cuneyt Tas, 2000).

Hydroxyapatite has been used widely in recent years for medical purposes. According to (Zimmermann, Leblanc, Sheets, Fox, & Gatenholm, 2011), over 2 million bone grafts are carry out to cover healthcare resulting in total cost of approximately $15billion. This increment of demand has gain a lot of awareness towards hydroxyapatite in medical area. It shows that, hydroxyapatite prove to be viable solution in assisting bone related problem or disease.

Recently, a lot of research has been done in bone tissue engineering to develop a high quality biological apatite substitute. The study was done to acquire a new technique for bone grafting to replace traditional graft such as autografts and allografts. These old techniques has bring a lot of problem like addition surgical pain and natural body rejection. Nevertheless, construction of alternative material that has the same properties as natural bone has been explore. (Yin et al., 2011)

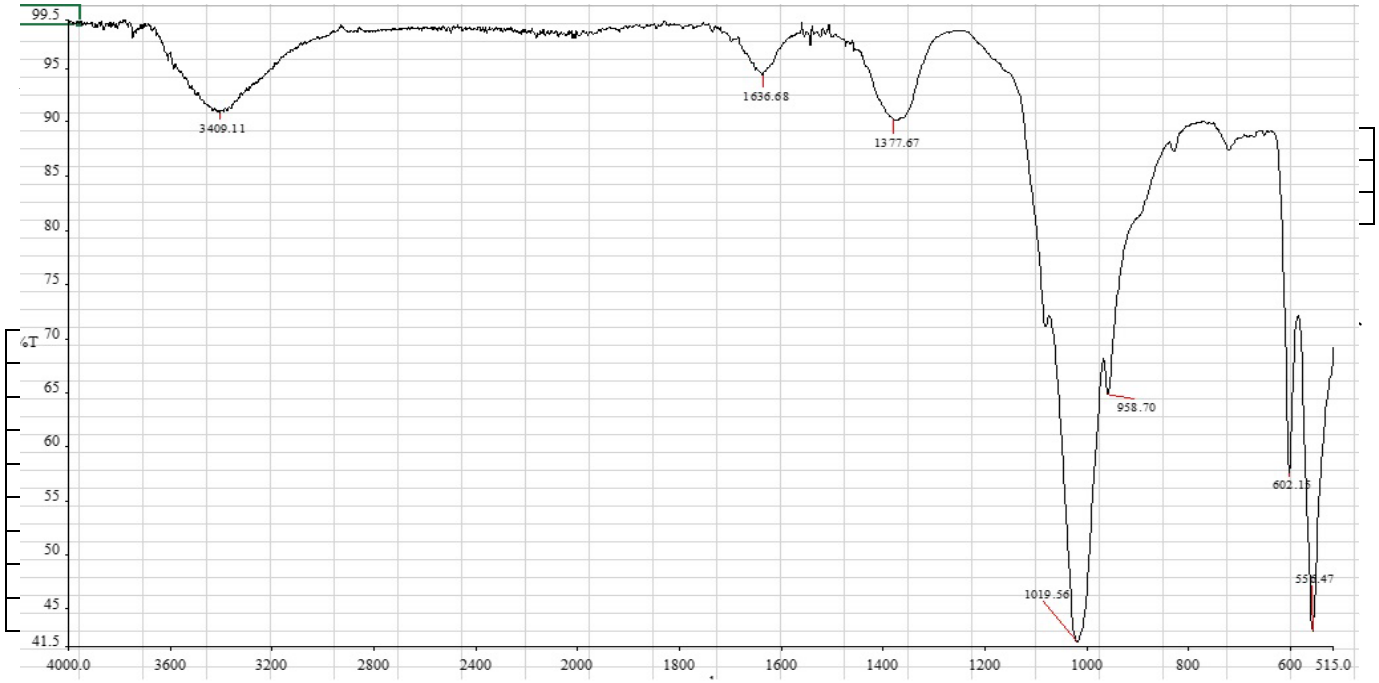
(Leena et al., 2016) claimed that hydroxyapatite is more reliable than biological apatite that calcium and carbonate deficient as well as variation of Ca/P molar ratio from the stoichiometric hydroxyapatite due to plentiful of substitutions and vacancies (traces of CO32-, Cl-, Na+, K+ and Mg+ ions). The nanosize of hydroxyapatite (70nm wide, 5nm thick and 100nm long) and its function has been consider as one of the most compatible substitute material for hard tissue repair and regeneration.

Hydroxyapatite popularly synthesis in biomimetic solution or simulated body fluid (SBF). This production has shown that it is not compatible to produce without the formation surrounding fibrous tissue. (Kokubo & Takadama, 2006) has develop a bone-bonding material which have essential requirement to produce hydroxyapatite in artificial living body, simulated body fluid. The vivo bioactivity can be predicted from the apatite formation in simulated body fluid solution due to its similarity to the human body.

The production of hydroxyapatite lately gain attention to solve bio-orthopedic problems. However, elevated processing conditions such as higher temperature have been report to produce hydroxyapatite with properties different from biological apatite. The process also led to the production of stoichiometric hydroxyapatite which lacks of usual inorganic constituent ions present in the bone mineral. (Leena et al., 2016)

There are few drawback that hydroxyapatite synthesis will suffer such as high pH that will form Ca-deficient HAp and high calcined temperature that will synthesis crystalline HAp. (Ben-Arfa, Salvado, Ferreira, & Pullar, 2017). To overcome this limitation, the synthesis of hydroxyapatite involves the use of simulated body fluid with controlled parameter to growth hydroxyapatite at body temperature & physiological pH. Subsequently, the synthesis technique use is precipitation method to produce a bone-line apatite. This study has led the synthesis of hydroxyapatite more beneficially in production and quality.

phate powder than can be completes in less than 20 minutes.



In simulated body fluid preparation, 8 types of chemical was dissolve orderly into 700ml of deionized water as shown in table 3. These chemical was added in order one by one after it dissolve completely.

Next, in the preparation of 1 L of SBF solution, a total of 44 ml of 1M HCl solution was added for pH adjustment where the 39 ml of the total solution was added before the 6th reagent (CaCl2.2H2O) to avoid turbidity. This process is to ensure there is no other activity during SBF preparation. Following the addition of 8th reagent, $(CH2OH)_3CNH_2$, the temperature of the process was increase from ambient temperature into 37°C.

Later, the solution was titrated with 1M HCl to a pH of 7 at 37°C. The leftover amount of HCl was used to titrate to avoid turbidity. During the titration process, the solution was continuously diluted with consecutive additions of deionized water to make the final volume equal to 1L.

B. Ion concentration of SBF solution

The ion concentration of simulated body fluid was determined by using UV spectrophotometer. Two type of ion are checked which are Na^+ and Cl^-

Table 3: Ion concentration of SBF

Ion	According to (Kokubo & Takadama, 2006) (mM)	Human Plasma (mM)
Na^+	142.0	142.0
Cl^-	147.8	103.0

These ion were diluted with water before scan through the UV spectrophotometer. It was diluted 1:10 to water for 4 dilution. This process was to observe the present of specific ion inside the simulated body fluid solution.

C. Synthesis of hydroxyapatite in SBF

2 chemicals as shown in table 4 were used to dissolve in SBF solutions. (Leena et al., 2016) observed that as soon as both reagents were dissolve in SBF, a slight turbidity was

sample was analyse by FTIR over the range between 4000 and 400cm-1 at 2cm-1 resolution averaging 132scans.

Figure 3: FTIR patterns of 800°C calcined HAp sample

III. RESULTS AND DISCUSSION

A. Ion concentration in simulated body fluid

In this study, the starting chemical at 4th order number was change from $K_2HPO_4.3H_2O$ to $Na_2HPO.2H_2O$. A significant improvement of Cl^- and HCO_3^- can be observe by changing the chemicals. Cl^- ion concentration was determined by using UV spectrophotometer and a significant changes can be observed where the result was almost identical to human plasma. However, the lack of equipment in this faculty to observe the HCO_3^- ion, the result was expected to be as stated from (Cuneyt Tas, 2000) where the ion concentration of HCO_3^- was increase from 4.2 mM to 27.0 mM which is exactly the same concentration as human plasma as shown in table 2.2 below. This changes generally affects the quality of SBF where the present SBF work is more reliable than previous study. He also says that the human plasma status is generally the best condition to have. Consequently, this similarity will affects the HAp production that contain the same nutrient as the human body.

Table 2.2 Ion concentration in SBF solution

Ion	According to (Kokubo & Takadama, 2006) (mM)	Result (mM)	Human Plasma (mM)
Na^+	142.0	142.0	142.0
Cl^-	147.8	123.0	103.0
HCO_3^-	4.2	27.0 (expected)	27.0
K^+	5.0	-	5.0
Mg^{2+}	1.5	-	1.5
Ca^{2+}	2.5	-	2.5
HPO_4^{2-}	1.0	-	1.0
SO	0.5	-	0.5

B. XRD Analysis

HAp sample from the calcination process at 800°C, 2hr were taken to analyse the phase purity of HAp by using XRD. From the figure 2 below, the peak can be observed at 28°, 31-33°, 39°, 46° and 49° for 2θ as the evidence of HAp crystal structure. The highest peak at the range of 31° to 33° shows a well-formed crystalline of HAp. This crystallinity of HAp depends on the process condition such as process temperature during SBF preparation and calcination factor.

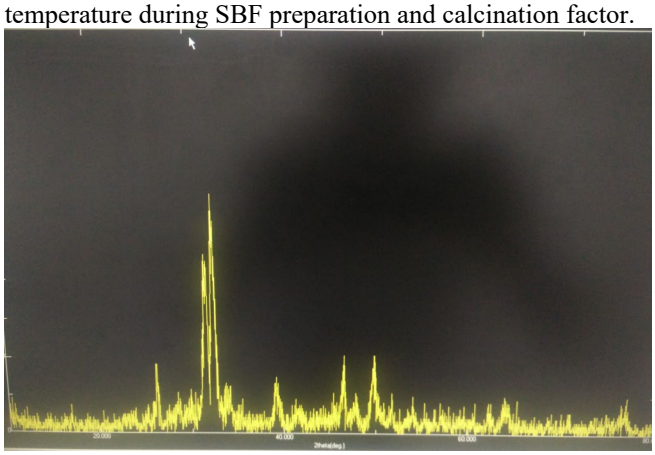


Figure 2: XRD patterns of 800°C calcined HAp sample

C. FTIR Analysis

HAp sample from the calcination process 800°C, 2hr were taken to analyse the functional group by using FTIR spectrophotometer. In figure 3 above, the image of FTIR spectra after the calcination at 800°C has shown significant build-up where it shows all the characteristic peak of HAp which the Ca and P precursor completely reacted. From the figure, few peak was observed with functional group such as adsorbed water (H_2O), phosphate (PO_4), carbonate (CO_3^{2-}) and hydroxyl (OH^-). The broad peak at 3409.11cm^{-1} observed to be formation of calcium phosphate at 0hr instead of HAp. Next, the presence of carbonate functional group can be observed at the peak of 1377.67 cm^{-1} and 958.70 cm^{-1}. According to (Landi, Celotti, Logroscino, & Tampieri, 2003) the presence of the carbonate peaks indicates the formation of B type HAp from the PO_4^{3-} and CO_3^{2-} anions. The major component of this functional group, PO_4^{3-} was observed at peak 1019.56cm^{-1}, 958.7cm^{-1}, 602.15cm^{-1} and 556.47cm^{-1}. The result shows that at 0hr of incubation, the calcium phosphate were formed instead of HAp formation. The large formation of calcium phosphate at early stage indicates the incubation time required for better HAp formation must be higher than 2hr as in this experiment.

IV. Conclusion

This study was to approach a method that produce bone-like biomimetic technique that synthesis a bone like-apatite. By using precipitation method, the Ca and P precursor was optimized decomposition of hydroxyapatite crystals by modification of ion concentration in simulated body fluid solution. HAp was produce in SBF solution and dried at 80°C for 1 day and calcined at 800°C for 2hr. HAp was deposited after 2hr of calcination where result expose that some calcium phosphate were produce during 0hr incubation. In this study, the hydroxyapatite was produce as B type carbonated HAp with well-formed crystallinity and shown significant build-up where all the characteristic peak of HAp which the Ca and P precursor completely reacted. However, the incubation period of calcination during HAp formation was not enough due to presence of calcium phosphate. This bone-like apatite can be used as material in bone structure and any medical area.

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